FUNDAMENTAL AND APPLIED TOXICOLOGY 19, 133–140 (1992)

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Fourteen-Day Inhalation Study in Rats, Using Aged and Diluted Sidestream Smoke from a Reference Cigarette[1]

I. Inhalation Toxicology and Histopathology

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Received October 9, 1991; accepted January 14, 1992

Fourteen-Day Inhalation Study in Rats, Using Aged and Diluted Sidestream Smoke from a Reference Cigarette. I. Inhalation Toxicology and Histopathology. COGGINS, C. R. E., AYRES, P. H., MOSBERG, A. T., OGDEN, M. W., SAGARTZ, J. W., AND HAYES, A. W. (1992). *Fundam. Appl. Toxicol.* 133–140.

Sprague–Dawley rats were exposed 6 hr per day for 14 consecutive days to aged and diluted sidestream smoke (ADSS), used as a surrogate for Environmental Tobacco Smoke (ETS), at concentrations of 0.1 (typical), 1 (extreme), or 10 (exaggerated) mg of particulates per cubic meter. Animals were exposed nose-only, inside whole-body chambers, to ADSS from the 1R4F reference cigarette. End-points included histopathology, CO-oximetry, plasma nicotine and cotinine, clinical pathology, and organ and body weights. The only pathological response observed was slight to mild epithelial hyperplasia and inflammation in the most rostral part of the nasal cavity, in the high-exposure group only. No effects were noted at medium or low exposures. The minimal changes noted were reversible, using a subgroup of animals kept without further treatment for an additional 14 days. Overall, the end-points used in the study demonstrated that there was no detectable biological activity of ADSS at typical or even 10-fold ETS concentrations and that the activity was only minimal at very exaggerated concentrations (particle concentrations 100 times higher than typical real-world concentrations). © 1992 Society of Toxicology.

Numerous statements have been made in the scientific literature on the biological activity of environmental tobacco smoke (ETS: Department of Health and Human Services, 1986; National Research Council, 1986). This study was designed to obtain information on the effects seen in animals exposed to aged and diluted sidestream smoke (ADSS), at concentrations equivalent and higher than those reported for ETS in the field. Measurements of the latter vary significantly and usually include contributions of suspended particulates other than ETS (DHHS, 1986). Consequently, the ADSS target concentrations were chosen as 0.1, 1, and 10 mg/m^3. Respectively, these correspond to typical, extreme (or "worst-case"), and exaggerated field measurements (Oldaker *et al.*, 1990).

The primary end-point of this study was the histopathology of the respiratory tract and related organs, as assessed by a pathologist with experience in rodent inhalation studies with cigarette smoke. The aims were to determine whether biological responses could be observed after exposure to ADSS at different concentrations, what these observed responses were, and the no-observed-effect-level (NOEL) for the study. An additional objective was to study the reversibility of any changes observed after exposure to ADSS. To study this recovery process, subgroups of animals were kept for an additional 2 weeks without treatment, at the end of the 14-day exposure.

Other end-points included DNA adducts and alveolar macrophage cytogenetics; these are reported separately (Lee *et al.*, 1992).

MATERIALS AND METHODS

Experimental design. The experimental design was based on published guidelines (OECD, 1981). Three groups of animals were exposed to ADSS; there was a sham-exposed group exposed only to filtered air. Animals were exposed to smoke inside whole-body chambers, using nose-only restraint tubes. A further group of animals was kept as chamber controls (without tube restraint) and as sentinels for the detection of disease. There were 48 animals per sex in each of the five groups. Animals were exposed 6 hr per day for 14 consecutive days. The groups were "stagger-started" at intervals of 1 day. Animals in satellite groups were kept for a further 14 days without treatment to assess reversibility.

Experimental animals. A total of 560 (280 male and 280 female) animals, weighing 125–150 g was purchased from Charles River Laboratories (Raleigh, NC). Animals were housed individually in transparent polycarbonate cages and acclimated to laboratory conditions for 14 days prior to the first exposure.

The Sprague–Dawley rat (Crl:CD/BR, VAF Plus) was chosen as the experimental animal because it has frequently been used in inhalation studies

[1] Presented at the 1992 Annual Meeting of the Society of Toxicology, Seattle, WA.

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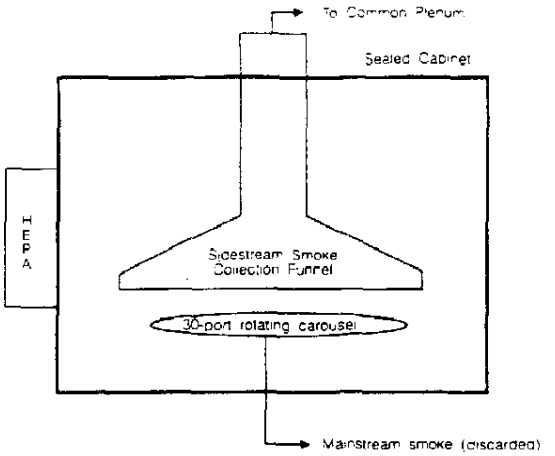

FIG. 1. Apparatus used to collect sidestream smoke from reference cigarettes.

and there is a large amount of background inhalation data available in the scientific literature.

Within 5 days of delivery, 5 animals per sex were randomly chosen and killed for collection of sera, which was tested for the following antibodies to disease: Reovirus Type 3, cilia associated respiratory bacillus, Kilham's rat virus, Toolan's H-1 virus, pneumonia virus of mice, Sendai, rat coronavirus/sialodacryoadenitis virus, lymphocytic choriomeningitis virus, and *Mycoplasma pulmonis*. Antibody testing was made on sera obtained from 5 animals per sex at the beginning and end of the inhalation part of the experiment, and at reversibility (total of 20 animals per sex). The lungs from the sentinel animals were taken and examined histopathologically to ascertain health status.

Within a week of delivery, the animals were allocated into five groups each of 48 animals per sex, such that the body weights in the groups were as homogeneous as possible. The mean (±SD, $n = 240$) weight of males at randomization was 142.1 ± 7.9 g; in females the mean was 148.4 ± 7.9 g.

During the week after allocation into groups, animals were tail-tattooed (Animal Identification and Marking Systems, Piscataway, NJ) with their permanent identification number.

The animals were housed and cared for in accordance with the Animal Welfare Act of 1970 and amendments (Public Law 91-579), as set forth in CFR Title 9, Part 3 Subpart E, *Specifications for the Humane Handling, Care, Treatment, and Transportation of Warm-Blooded Animals Other Than Dogs, Cats, Rabbits, Hamsters, Guinea Pigs and Non-human Primates*. Reference was also made to the DHHS document *Guide for the Care and Use of Laboratory Animals* (NIH Publication 86-23).

Animals had unrestricted access to certified feed (Purina Rodent Chow No. 5002, presented as pellets) and distilled water. No feed was available during inhalation exposures. Feed was withheld overnight prior to necropsy. Chemical analyses of feed, water, or bedding were not performed, because it was deemed unlikely that contaminants would adversely affect the experiment.

Cigarettes. The 1R4F reference cigarettes were purchased from the Tobacco and Health Research Institute (Lexington, KY). A full description of the mainstream, sidestream, and ETS chemistry of the 1R4F cigarette has been given elsewhere (R. J. Reynolds, 1988).

Aerosol generation apparatus. A 30-port smoke generator (CH Technologies, Westwood, NJ) similar to that described by Baumgartner and Coggins (1980) and by Ayres *et al.* (1990) was fitted with an aluminum cone (Fig. 1) for collection of sidestream smoke (SS). The smoke generator was placed in a sealed cabinet with a HEPA filter attached. Mainstream smoke was generated under Federal Trade Commission conditions (except butt length: seven puffs were taken instead of burning to a fixed butt length) and was discarded. Sidestream smoke was drawn from the cone into a common plenum, using 3-in.-diam. (76.2 mm) PVC tubing throughout. Different amounts of SS were drawn from the plenum for each chamber and mixed with dilution air drawn from the room through HEPA filters (Fig. 2). Room air was HEPA-filtered "upstream." The sham and sentinel animals were kept in chambers that were not attached to the plenum.

Animal exposure apparatus. The whole-body inhalation chamber has been described previously (Moss *et al.*, 1982) and is available commercially (Lab Products, Maywood, NJ). Each chamber was operated at a flow rate of 16 cubic feet/min (in excess of 15 air changes/hr).

The conical nose-only restraint tubes (Baumgartner and Coggins, 1980) are also commercially available (CH Technologies, Westwood, NJ); they were used to minimize contamination of the pelt with deposited ADSS which could then be absorbed dermally or ingested during preening (Langard and Nordhagen, 1980). Tubes for males were 73 mm in diameter and 263 mm long; for females the diameter was 62 mm and the length 168 mm. Both tube types had inlets 22 mm in diameter. The ventilation slots on the restraint tubes were covered with duct tape.

On exposure days, individual animals were taken from their cage in the chamber, placed inside a nose-only restraint tube, and replaced in the tube into the same cage. The orientation of the tube was such that urine and feces passed from the tube, through the wire floor of the cage, and onto paper-lined catch pans placed under each of the trays.

Daily characterization of inhalation exposures. During animal exposures, probes were used to monitor the aerosol presented. This monitoring was by collection of aerosol on glass-fiber pads followed by gravimetric determination of collected particulates using Cahn C-31 microbalances (Cahn, Cerritos, CA). The RAM-1 instrument was used to give an on-line estimate of particulate concentrations.

The main analytical instrument used for carbon monoxide (CO) and carbon dioxide (CO_2) was the Horiba PIR-2000 (Horiba Instruments, Inc., Irvine, CA), calibrated daily with certified gas mixtures (AIRCO Welding Supply, Greensboro, NC). Oxygen concentrations (%) were monitored by a Horiba PMA-200 instrument, also calibrated with a certified gas mixture. Very low concentrations of CO were measured with the Miran 80 gas analyzer (Foxboro Instruments, S. Norwalk, CT). Data from the on-line instruments were logged manually every 60 min.

Measurement of nicotine and 3-ethenylpyridine was by gas chromatography with thermionic-specific detection (Ogden, 1991). Chamber atmo-

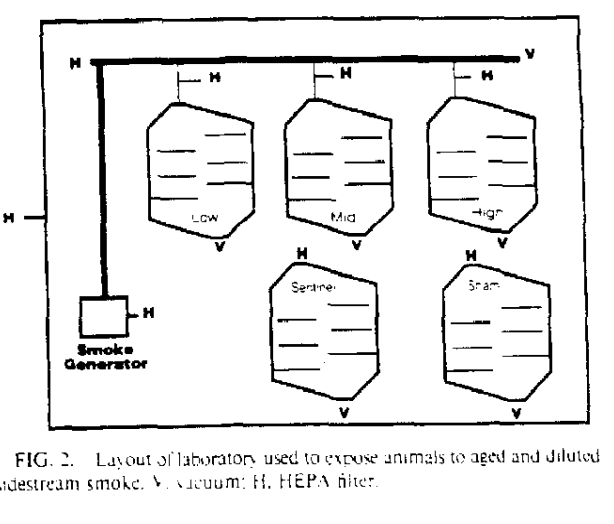

FIG. 2. Layout of laboratory used to expose animals to aged and diluted sidestream smoke. V, vacuum; H, HEPA filter.

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spheres were sampled with XAD-4 sorbent tubes (SKC, Inc., Eighty-Four, PA) which were extracted for analysis with ethyl acetate containing 0.01% triethylamine (Ogden, 1989).

Solanesol measurements were made by liquid chromatography with UV detection at 205 nm (Ogden and Maiolo, 1990). Following gravimetric determination of particulates, the glass-fiber pads were extracted for analysis with 3 ml methanol.

Measurement of particle size distribution was made three times during the study, using a Mercer-style cascade impactor (Mercer *et al.*, 1970; In-Tox Products, Albuquerque, NM). The impactor had cut-off diameters in the range of 0.4–2.5 μm under the conditions of use (1.2 liters/min); calculation of mass median aerodynamic diameter was by probit analysis. The coverslips (uncoated) used to collect the aerosol for impactor analysis were weighed using Cahn C-31 microbalances.

Air temperature and RH of the aerosol were measured with a condensation dew point hygrometer (Model 1100 DP, General Eastern Instrument Co., Watertown, MA).

Clinical observations. Animals were inspected visually for signs of overt toxicity as they were being transferred from their cages to the restraint tubes, and when being transferred back to their cages. More detailed clinical observations were made on each animal once every 4 days, before the exposure and within 2 hr of the end of the exposure.

Body weights. Individual body weights were determined within 48 hr of receipt, at randomization, and every 4 days thereafter, using Mettler PM 2000 balances.

Dosimetry. Blood samples were obtained after 6 hr of smoke exposure, on different exposure days throughout the experiment. Blood was drawn from the retro-orbital sinus, using anesthesia with 70% CO_2 in air and heparinized micropipettes, and held on ice in plastic cuvettes containing disodium edetate during the time between sampling and analysis.

Blood COHb concentrations were determined on 0.5 ml of the total sample, using a Model 482 CO-Oximeter (Instrumentation Laboratories, Hartford, CT). Subsamples of the blood collected for COHb were taken for the determination of plasma nicotine and cotinine. The latter analyses were performed by an ELISA method.

Necropsy. Animals selected at random were killed on the day following their last exposure, and the time interval was recorded. Feed was not available to the animals during this time interval.

At necropsy, animals were weighed and then killed by first anesthetizing with 70% CO_2 in air and then exsanguination via the vena cava prior to cessation of heartbeat. Blood samples for the various assays to be performed were collected from the vena cava.

Animals were subjected to a complete gross examination in the presence of a board-certified veterinary pathologist, with special attention paid to the respiratory tract.

Clinical pathology. The following assays were performed on whole blood obtained at each necropsy: red blood cell count, hemoglobin, hematocrit, mean red cell volume, mean red cell hemoglobin, mean red cell hemoglobin concentration, white cell count, differential white cell counts, reticulocyte counts, and platelet count. The anticoagulant Na_2-EDTA was used; standard hematological methods were used.

The following assays were performed on serum obtained from animals at each necropsy: calcium, phosphorus, chloride, sodium, potassium, glucose, alanine aminotransferase, aspartate aminotransferase, γ-glutamyl transpeptidase, urea nitrogen, albumin, creatinine, total bilirubin, total cholesterol, triglycerides, and total protein. The time of blood sampling was recorded. Sure-Sep II serum separators (Organon-Teknika, Durham, NC) were used to minimize hemolysis; the time between blood collection and serum collection was kept as short as possible. Standard analytical methods were used.

Organ weights. The lungs (complete with trachea but excluding the larynx), brain, liver, testes (pair), kidneys (pair), and heart (excluding major vessels) were weighed at each necropsy using Mettler PM 460 balances. Organ weights and the (fasted) body weight recorded immediately before death were used to calculate organ:body weight ratios. The time from removal of the organ until weighing was minimized and tissues were kept in saline until they were weighed.

Tissue collection. Tissues were removed from each animal and fixed in 10% neutral buffered formalin (NBF), at a volume dilution of 1 part tissue to at least 15 parts formalin (Feldman and Seely, 1988). The fixative contained 20 ml of 1% eosin per 20 liters of 37% formalin, as a precaution to identify the fluid as fixative. Lungs were infused with NBF at a volume that ensured proper fixation. The trachea was ligated after inflation.

The following tissues were collected: adrenals, aorta, bone (sternum, femur), brain, cecum, colon, cranium, duodenum, epididymides, esophagus, eyes/optic nerve, heart, ileum, jejunum, kidneys, larynx, liver, lungs, lymph nodes, mammary glands, nasopharynx, nose/turbinates, ovaries, pancreas, parathyroid, pituitary, prostate, rectum, salivary gland, seminal vesicle, skeletal muscle (thigh), skin (abdominal), spinal cord (lumbar), spleen, stomach, tail, testes, thymus, thyroid, tongue, trachea, urinary bladder, uterus, Zymbal's gland.

Histopathology. Respiratory tract tissues (nasal passages, larynx, trachea, conducting airways, deep lung), heart and related lymph nodes (thymic and peribronchial), and gross changes were examined in each of 28 animals per sex randomly allocated to histopathology in each of the four exposure groups (20 for the 14-day necropsy and 8 for the reversibility necropsy).

The nasal tissues were cut at three different locations to obtain representative sections of the different epithelia, as described previously (Young, 1981). The lungs were sectioned to provide a section along the main stem bronchus of each lung lobe. A precise anatomical site for cutting the larynges is required; serial-step sections were taken to reach this site (Burger *et al.*, 1989; Sagartz *et al.*, 1992).

Tissues were stained with hematoxylin and eosin (H&E); duplicate slides of a representative section of the anterior nasal tissues, larynx, lung, and trachea were stained with periodic acid–Schiff/Alcian blue to facilitate evaluations of mucus-secreting cells. Tissues were read by an ACVP board-certified veterinary pathologist, with knowledge of the treatment groups.

Statistical analyses. Statistical evaluations were made using Bartlett's test of homogeneity of variance, followed by analysis of variance techniques. The statistical evaluation of incidence and severity data for histopathology was made by the Kolmogorov–Smirnov test (Siegel, 1956). Statistical tests were carried out to 5%, two-sided criteria.

RESULTS

Inhalation Exposures

The mean concentrations (±SD, n = 14) of wet total particulate matter (WTPM) for the low-, medium-, and high-exposure groups were 0.09 ± 0.01, 1.08 ± 0.08, and 9.79 ± 0.59 mg/m^3, respectively. Figure 3 shows the within- and between-day variation in WTPM concentrations. These WTPM exposures resulted in CO concentrations (ppm) of 3.61 ± 0.81, 11.3 ± 1.6, and 57.0 ± 3.8.

Nicotine concentrations (±SD, n = 14) at the medium and high exposures were 252 ± 57 and 1708 ± 346 μg/m^3. Although nicotine could be detected in the low-exposure chambers, the values were unexpectedly low; the mean was 0.91 ± 0.28 μg/m^3. The ratios of nicotine to WTPM were 1.01 ± 0.27, 23.4 ± 5.1, and 17.4 ± 3.4% for low, medium, and high exposures, respectively. The values for 3-ethenylpyridine at the low, medium, and high exposures were 1.30 ± 0.34, 36.0 ± 3.0, and 242 ± 26 μg/m^3; these data resulted in ratios of 3-ethenylpyridine to nicotine of 147 ± 36, 14.8 ± 3.3, and 14.7 ± 3.4% for low, medium, and high exposures, respectively.

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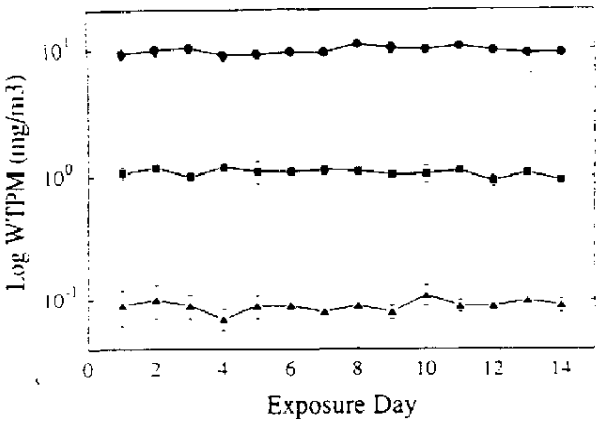

FIG. 3. Variation in daily concentrations of wet total particulate matter in aged and diluted sidestream smoke presented to experimental animals. Means ± standard deviations. Targets were 0.1, 1, and 10 mg/m^3.

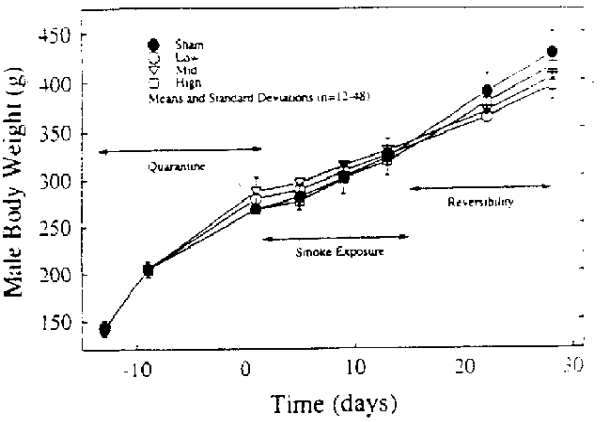

FIG. 4. Change in body weight of male animals.

Solanesol concentrations (±SD, n = 14) in the low, medium, and high exposures were 1.93 ± 0.24, 25.1 ± 2.3, and 186 ± 18 μg/m^3, respectively; the ratios of solanesol to WTPM were 2.16 ± 0.32, 2.33 ± 0.11, and 1.90 ± 0.11%.

None of the above analytes could be detected at significant concentrations in the sham chamber or in the exposure room. In the exposure room, the mean CO_2 concentration (±SD, n = 16) was 403 ± 20 ppm; in the sham chamber the mean (±SD, n = 14) concentration was 1307 ± 58 ppm. The mean concentrations (±SD, n = 14) in the low, medium, and high chambers were 1522 ± 101, 1458 ± 166, and 1842 ± 100 ppm, respectively.

Particle size distributions at the medium and high exposures were similar, with the average values (three observations) being 0.42 and 0.29 μm, respectively. The standard geometric deviations were 1.52 and 1.80. Insufficient material was obtained during the 6 hr of the low exposure for determination of particle size distribution.

In-Life Observations

There were no treatment-related clinical signs or mortalities and the serology results were negative. Animals in the smoke-exposed groups showed body weights that were similar to those in the sham group (Figs. 4, 5).

Blood COHb concentrations at the end of the exposures were negligible in the sham low-exposure groups. The means for the medium and high groups (sexes combined, ±SD, n = 30) were 0.91 ± 0.46 and 5.80 ± 0.94%, respectively.

The ELISA assay had limits of detection (LOD) of 4.5 ± 3.3 ng/ml for nicotine and 6.9 ± 7.6 ng/ml for cotinine. The sham and low-exposure groups had plasma nicotine concentrations at or below the LOD; the medium-exposure group had plasma nicotine concentrations above the LOD but below the limit of quantification. The mean plasma nicotine for the high exposure (sexes combined, ±SD, n = 30) was 43.6 ± 13.5 ng/ml.

The sham and low-exposure groups had plasma cotinine concentrations at or below the LOD. The means for the medium and high exposure (sexes combined, ± SD, n = 29) were 43.2 ± 15.7 and 323 ± 161 ng/ml, respectively.

Necropsy Data

There was no effect of exposure on terminal body weight; the mean values (± SD, n = 21–24) in males for sham, low, medium, and high exposures at the 14-day necropsy were 287 ± 18, 299 ± 25, 300 ± 21, and 288 ± 17 g, respectively. In females, the means were 212 ± 12, 211 ± 15, 210 ± 15, and 204 ± 11 g. At the recovery necropsy, the means (±SD, n = 12–16) for males were 380 ± 50, 363 ± 34, 368 ± 39, and 377 ± 46 g for the sham, low, medium, and high exposures, respectively; for the females, the values were 251 ± 15, 259 ± 14, 250 ± 20, and 247 ± 24 g.

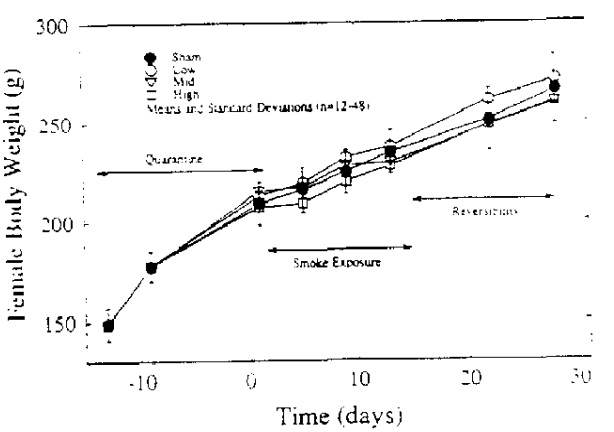

FIG. 5. Change in body weight of female animals.

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FIG. 6. Low-power view of a transverse section of the rat nasal passages, immediately caudal to the incisor teeth. The arrow marks the position of the change shown in Figs. 7 and 8. H&E; original magnification, ×45.

There were no significant differences between the groups for any clinical pathology parameters at either of the necropsies.

There were no treatment-related gross observations at either necropsy; there were no differences in organ weights.

Histopathology

In the Nasal I section (transverse, immediately caudal to the incisor teeth), there was *chronic active inflammation*, in the high-exposure groups only. This change mainly involved the dorsal nasal conchae (nasoturbinates) and the adjacent wall of the middle meatus (Fig. 6). In this lesion, the inflammatory infiltrate consisted of lymphohistiocytic cells admixed with small numbers of polymorphonuclear leukocytes. Figure 7 shows a high-power view of the tip of a nasal concha from a typical sham animal; Fig. 8 shows a similar region from a typical high-exposure-group animal. Figure 9 shows the distributions of the different severities of this change for the four groups. The distribution of the change in the high-exposure group was significantly different from that in the other groups; there were no such significant differences at the low or medium exposures.

The above lesion was typically found in association with, and in the same anatomic site as, *epithelial hyperplasia*. In this latter change, there was hypercellularity and thickening of the respiratory epithelium of the dorsal nasal conchae and the adjacent wall of the middle meatus. Figure 10 shows the

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FIG. 7. High-power view of the tip of a nasal concha (nasoturbinate) from a typical sham-exposed rat. The epithelium is two to three cell layers thick. H&E; original magnification, ×500.

distribution of the different severities of this change for the four groups. The distribution of the change in the high-exposure group was significantly different from that in the other groups; no such significant effect was noted at the low or medium exposures.

There were no other histopathological changes noted in animals killed at the 14-day necropsy, nor were any changes noted in the animals killed at the reversibility necropsy (i.e., the minimal changes noted at the end of the exposure were totally reversible).

DISCUSSION

The results obtained here are in agreement with earlier work (von Meyerinck *et al.*, 1989), where only a single concentration of ADSS was used (4 mg/m^3) for 10 hr/day. In this latter work, the main histopathological change noted was also in the rostral nasal cavity, a change which appears morphologically to be very similar to that noted in the high-exposure group in the present study, although the overall duration was much less (14 days instead of 90). It is thus possible that the change does not progress in subchronic exposures.

The analytical results described here show clearly that solanesol is a good marker for the particulate phase of ADSS, there being a constant ratio between solanesol measured by liquid chromatography and gravimetric estimates of WTPM

FIG. 8. High-power view of the tip of a nasal concha (nasoturbinate) from a typical high-exposure rat. The epithelium is five to six cell layers thick. Note the presence of an inflammatory infiltrate, consisting of lymphohistiocytic cells admixed with small numbers of polymorphonuclear leukocytes. H&E; original magnification, ×500.

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(mean ratio 2.1%). This ratio is in very good agreement with ratios for true ETS generated by human smokers in a controlled environment. Ratios of solanesol to particulates from the 1R4F cigarette in these environments range from 2.6 to 4.0% with a mean ratio of 3.2% (Ogden, unpublished data). The slightly lower ratios noted in this study can be attributed to the smoke generation and particulate analysis systems used. Sidestream smoke generated only by a smoking machine results in slightly lower solanesol concentrations than does human smoking which includes sidestream smoke and exhaled mainstream smoke (Ogden, unpublished data). In addition, particulates were determined as WTPM in this study which, by definition, includes water. Significant moisture condensation on the particles will deflate the solanesol/WTPM ratio and apparently does so at the high exposure used here. Measurement of ETS particulates is not appreciably affected by trapped water due to the high dilution of the smoke aerosol. Likewise, water does not appear to contribute significantly to the WTPM concentrations measured at the low and medium exposures.

The surprisingly low nicotine concentrations in the low-exposure chamber have not yet been explained. One hypothesis is that nicotine is selectively adsorbed on the large surface area of the chamber or the connecting plumbing. This reasoning is consistent with known adsorption characteristics of nicotine in a stainless-steel environmental chamber (Thorne *et al.*, 1986). Due to the unusual decay characteristics of nicotine in some situations, 3-ethenylpyridine (a product of nicotine combustion) is gaining acceptance as a tracer for ETS vapor phase (Ogden, 1991).

The exposure levels used in this study were carefully chosen with respect to typical concentrations in real-life environments. The low exposure used (target and actual particulate concentrations of 0.10 and 0.09 mg/m^3, respectively) is typical of exposure concentrations in public places where smoking is allowed without restriction. For example, average concentrations of tobacco smoke indicators in restaurants are 10.5 μg/m^3 nicotine; 2.5 μg/m^3 3-ethenylpyridine (Ogden, unpublished data); and 0.108 mg/m^3 particulates and 1.5 μg/m^3 solanesol (Oldaker *et al.*, 1990). These concentrations are equivalent to the concentrations generated in the low exposure (excepting the anomaly noted for the nicotine concentration). As an example of more extreme conditions, the highest concentration of solanesol ever measured in a real-life situation is 12.8 μg/m^3 (with an associated particulate concentration of 0.355 mg/m^3), recorded in a smoke-filled billiard parlor (Ogden and Maiolo, 1989). This concentration is approximately half that measured in the medium exposure of this study.

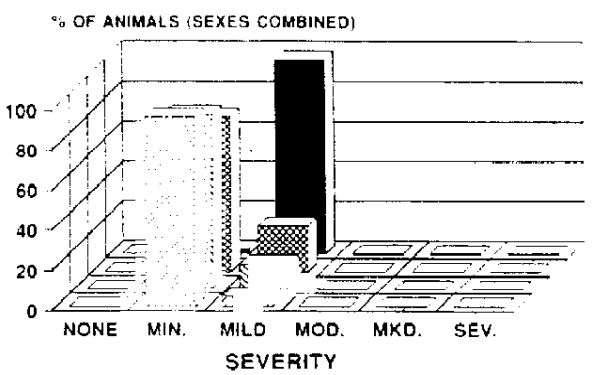

FIG. 9. Distribution of severities of chronic active subepithelial inflammation in Nasal I in the different groups at the 14-day necropsy (sexes combined). MIN., minimal change; MILD, mild change; MOD., moderate change; MKD., marked change; SEV., severe change. (□) Sham, (▨) low, (▩) medium, (■) high.

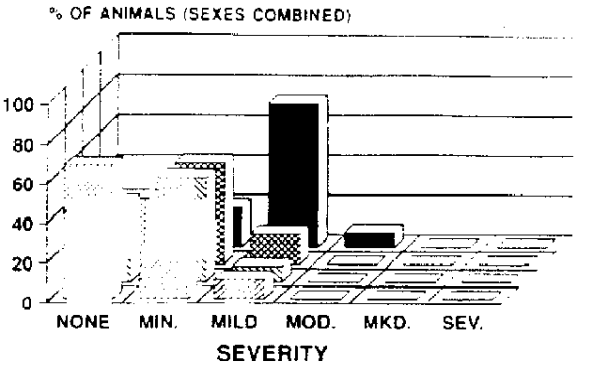

FIG. 10. Distribution of epithelial hyperplasia in Nasal I in the different groups at the 14-day necropsy (sexes combined). MIN., minimal change; MILD, mild change; MOD., moderate change; MKD., marked change; SEV., severe change. (□) Sham, (▨) low, (▩) medium, (■) high.

The results presented here show only a minimal effect of exposure to very high concentrations of aged and diluted sidestream smoke, the only effect being the completely reversible (in 14 days) changes seen in the rostral nasal cavity at the high exposure only. Since the concentrations of smoke used were gross exaggerations (100-fold) of any reasonable field situation for ETS (DHHS, 1986; Oldaker *et al.*, 1990), we conclude that ETS, at typical and maximal concentrations, is unlikely to have any significant toxicological activity in tests similar to those used here. The NOEL for the study is 1 mg/m^3.

ACKNOWLEDGMENTS

The authors thank Jerry Avalos, Jim Corn, Delma France, and Keith Shreve for performing the inhalation exposures; Jessica Baker for animal care; Katherine Maiolo for the nicotine, solanesol, and 3-ethenylpyridine measurements; Sharon Reynolds for serology and the COHb assay; Leroy Gerald for necropsy; Dr. Kuo-Mei Chang for the plasma nicotine analyses; and Mark Forsell and Arpad Musdarasz for the histology.

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PM3006447527

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